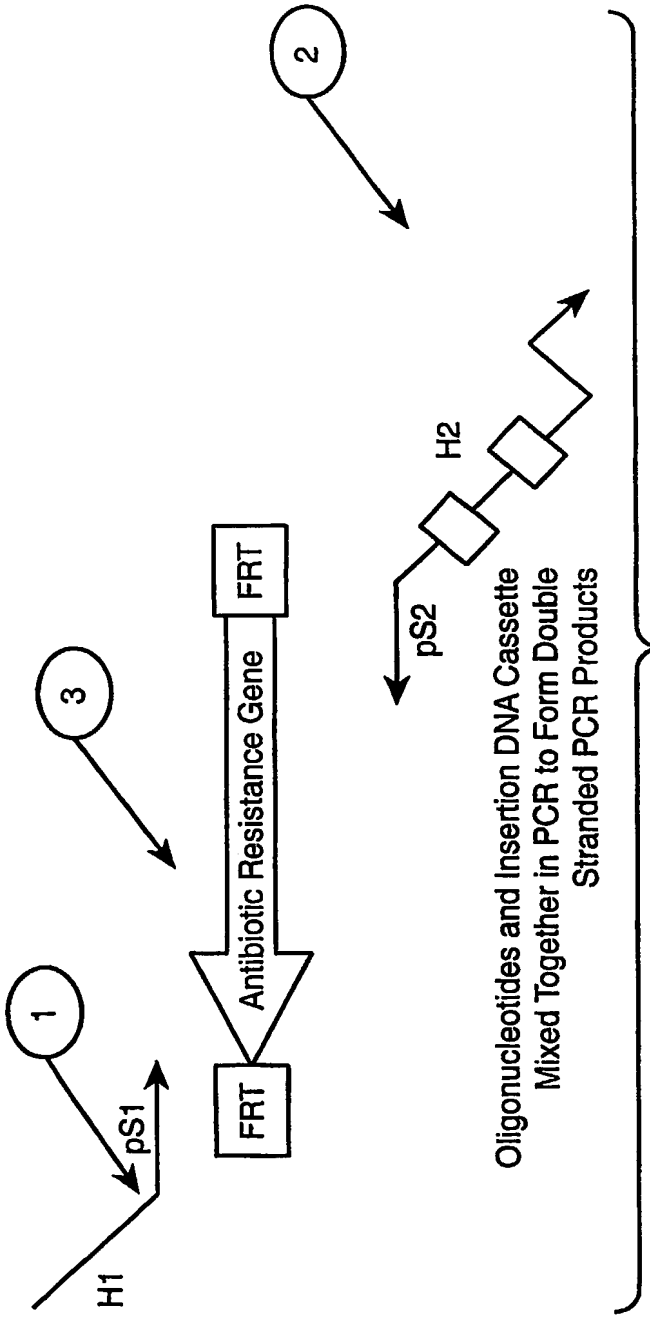
1
pS1
H1
3
FRT
Antibiotic Resistance Gene
FRT
2
H2
pS2
Oligonucleotides and Insertion DNA Cassette
Mixed Together in PCR to Form Double
Stranded PCR Products

FIG._1A

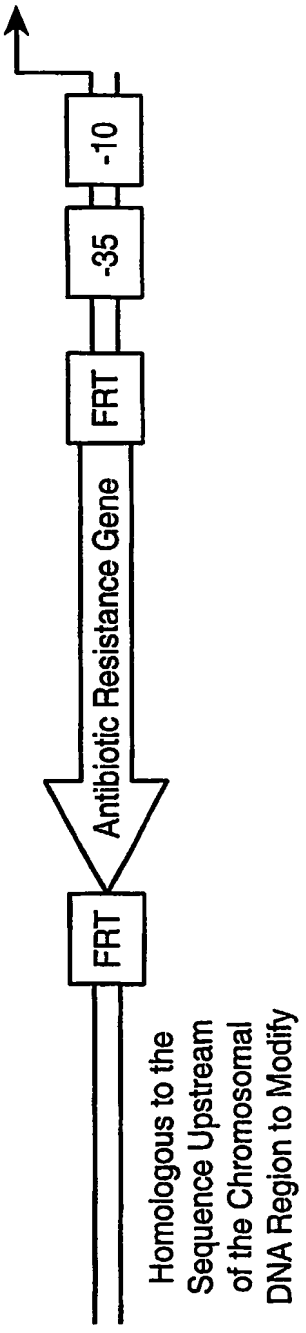
-10
-35
FRT
Antibiotic Resistance Gene
FRT
Homologous to the
Sequence Upstream
of the Chromosomal
DNA Region to Modify

FIG._1B

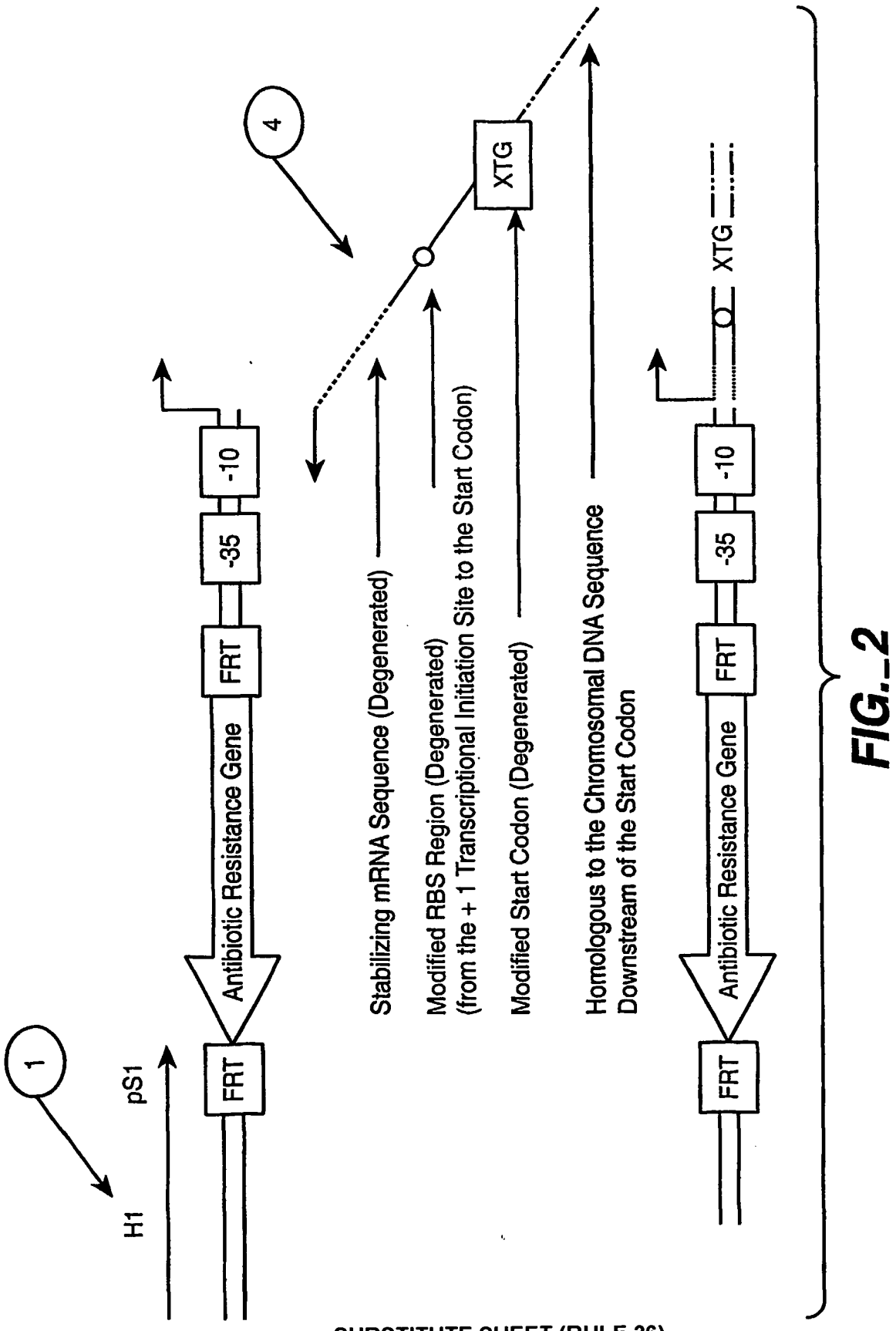
1
H1
pS1
FRT
Antibiotic Resistance Gene
FRT
-35
-10
4
XTG
Stabilizing mRNA Sequence (Degenerated)
Modified RBS Region (Degenerated)
(from the + 1 Transcriptional Initiation Site to the Start Codon)
Modified Start Codon (Degenerated)
Homologous to the Chromosomal DNA Sequence
Downstream of the Start Codon
XTG
-10
-35
FRT
Antibiotic Resistance Gene
FRT

FIG._2

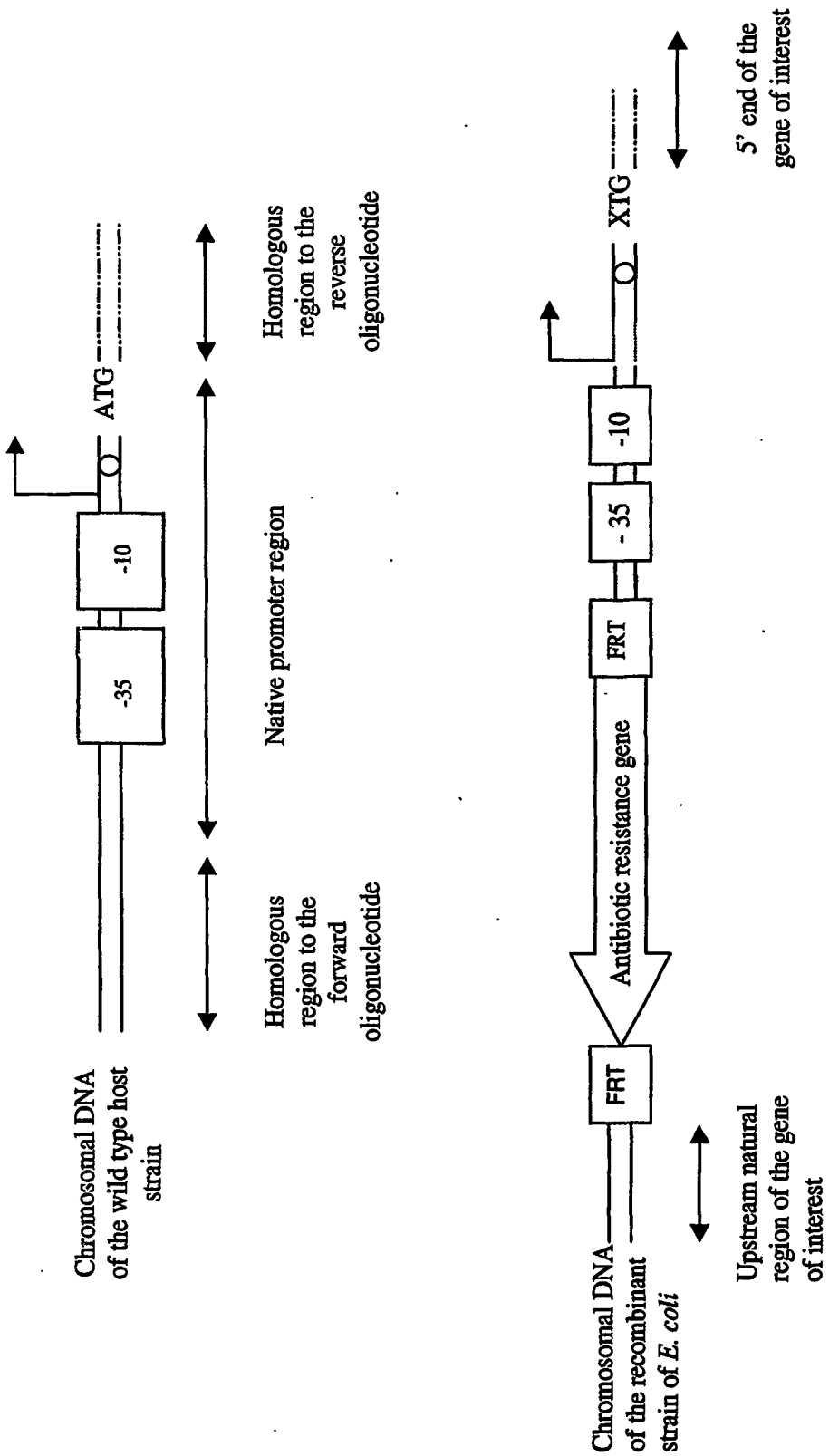
Chromosomal DNA of the wild type host strain
-35
-10
ATG
Native promoter region
Homologous region to the forward oligonucleotide
Homologous region to the reverse oligonucleotide
Chromosomal DNA of the recombinant strain of E. coli
FRT
Antibiotic resistance gene
FRT
- 35
-10
XTG
Upstream natural region of the gene of interest
5' end of the gene of interest

FIGURE 3

FIGURE 4

| | -50 | | -7 | +1 | +20 | |
|---|---|---|---|---|---|---|
| PH/E20 | AACTGCAAAAATAGT | **TTGACA** | CCCTAGCCGATAGGCTT | **TAAGAT** | GTACCCAGTTCGATGAGAGCGATAAC | (SEQ ID NO. 3) |
| PH207 | TTTTAAAAAATTCAT | **TTGCTA** | AACGCTTCAAATTCTCG | **TATAAT** | ATACTTCATAAATTGATAAACAAAAA | (SEQ ID NO. 4) |
| PN25 | TCATAAAAAATTTAT | **TTGCTT** | TCAGGAAAATTTTTCTG | **TATAAT** | AGATTCATAAATTGAGAGAGGAGTT | (SEQ ID NO. 5) |
| PG25 | TGAAAAATAAAATTC | **TTGATA** | AAATTTTCCAATACTAT | **TATAAT** | ATTGTTATTAAAGAGGAGAAATTAAC | (SEQ ID NO. 6) |
| PJ5 | ATATAAAAACCGTTA | **TTGACA** | CAGGTGGAAATTTAGAA | **TATACT** | GTTAGTAAACCTAATGGATCGACCTT | (SEQ ID NO. 7) |
| PA1 | TTATCAAAAAGAGTA | **TTGACT** | TAAAGTCTAACCTATAG | **GATACT** | TACAGCCATCGAGAGGGACACGGCGA | (SEQ ID NO. 8) |
| PA2 | CACGAAAAACAGGTA | **TTGACA** | ACATGAAGTAACATGCAG | **TAAGAT** | ACAAATCGCTAGGTAACACTAGACGC | (SEQ ID NO. 9) |
| PA3 | GGTGAAACAAAACGG | **TTGACA** | CACTGAAGTAAACACGG | **TACGAT** | GTACCACATGAAACGACAGTGAGTCA | (SEQ ID NO. 10) |
| PL | TTATCTCTGGCGGTG | **TTGACA** | TAAATACCACTGGCGGT | **GATACT** | GAGCACATCAGCAGGACGCACTGACC | (SEQ ID NO. 11) |
| Plac | TTAGGCACCCCAGGC | **TTTACA** | CTTTATGCTTCCGGCTGG | **TATGTT** | GTGTGGAATTGTGAGCGATAACAAT | (SEQ ID NO. 1) |
| PlacUV5 | CTAGGCACCCCAGGC | **TTTACA** | CTTTATGCTTCCGGCTGG | **TATAAT** | GTGTGGAATTGTGAGCGGATAACAAT | (SEQ ID NO. 12) |
| PtacI | TTCTGAAATGAGCTG | **TTGACA** | ATTAATCATCGGCTCG | **TATAAT** | GTGTGGAATTGTGAGCGGATAACAAT | (SEQ ID NO. 2) |
| Pcon | AATTCACCGTCGTTG | **TTGACA** | TTTTTAAGCTTGGCGGT | **TATAAT** | GGTACCATAAGGAGGTGGATCCGGCA | (SEQ ID NO. 13) |
| Pbls | TTTTTTCTAAATACA | **TTCAAA** | TATGTATCCGCTCATGA | **GACAAT** | AACCCTGATAAATGCTTCAATAATAT | (SEQ ID NO. 14) |

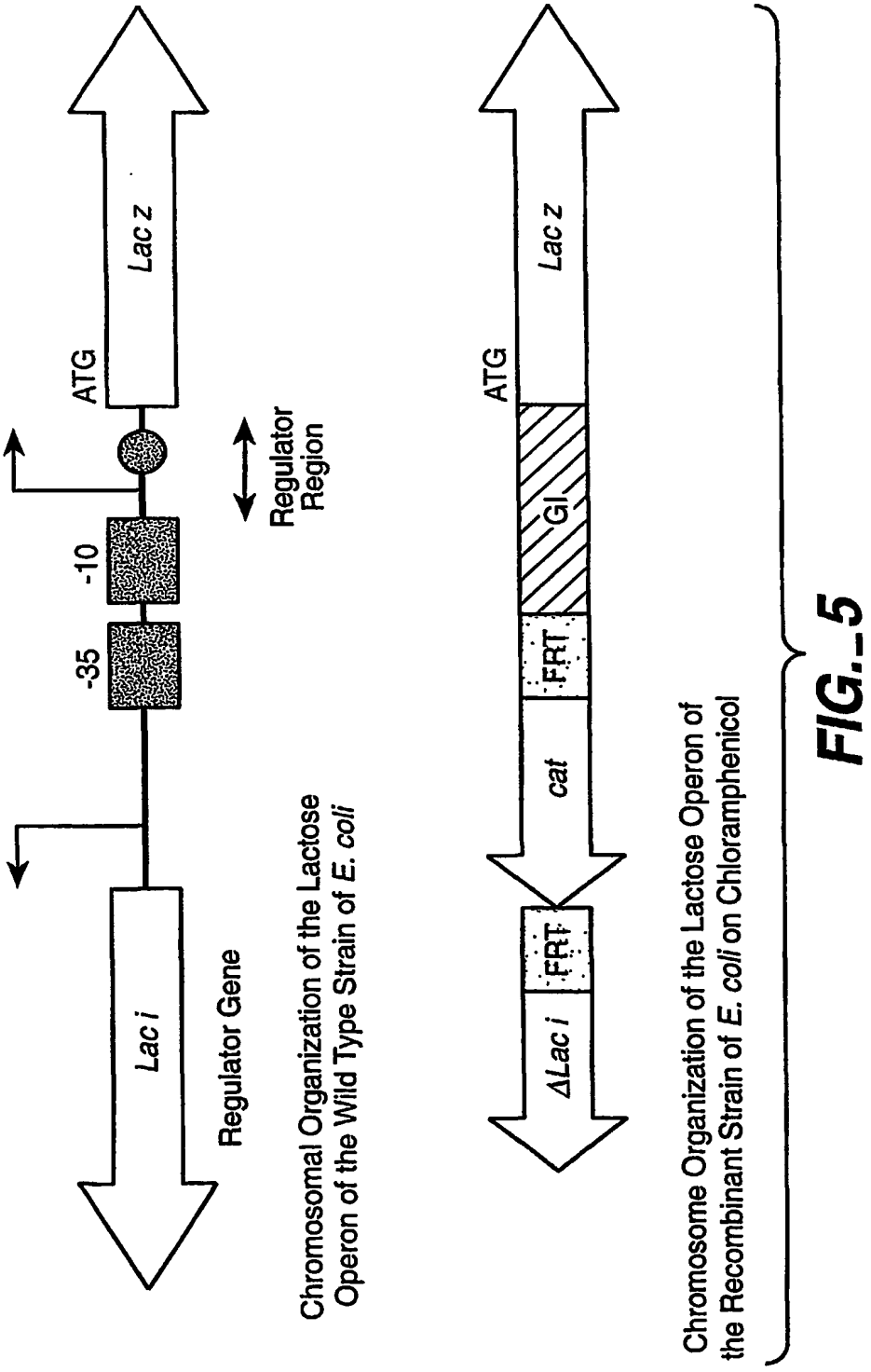
ATG
Lac z
Regulator Region
-10
-35
Lac i
Regulator Gene
Chromosomal Organization of the Lactose Operon of the Wild Type Strain of E. coli
ATG
Lac z
GI
FRT
cat
FRT
ΔLac i
Chromosome Organization of the Lactose Operon of the Recombinant Strain of E. coli on Chloramphenicol

FIG._5

FIGURE 6

***pLAC*** (SEQ ID NO. 18)

AGGC TTTACA CTTTATGCTTCCGGCTCG TATGTT GTGTGGA ATTGTGAGCGGATAACAATTTCACAC*AGGAAA*CAGCT ATGACC

**- 35** **-10** **RBS** **Start**

**1.6GI lacZ** (SEQ ID NO. 19)

GCCC TTGACA ATGCCACATCCTGAGCA AATAAT TCAACCACT AATTGTGAGCGGATAACAATTTCACAC*AGGAAA*CAGCT **ATGACC**

**-35** **-10** **RBS** **start**

**GI 1.6** (SEQ ID NO. 15)

GCCC **TTGACA** ATGCCACATCCTGAGCA **AATAAT** TCAACCACTAATTGTGAGCGGATAACA

**1.5GI lacZ** (SEQ ID NO. 20)

GCCC TTGACT ATGCCACATCCTGAGCA AATAAT TCAACCACT AATTGTGAGCGGATAACAATTTCACAC*AGGAAA*CAGCT ATGACC

**-35** **-10** **RBS** **start**

**GI 1.5** SEQ ID NO. 16

GCCC **TTGACT** ATGCCACATCCTGAGCA **AATAAT** TCAACCACTAATTGTGAGCGGATAACA

**1.20GI lacZ** (SEQ ID NO. 21)

GCCC TTGACG ATGCCACATCCTGAGCA AATAAT TCAACCACT AATTGTGAGCGGATAACAATTTCACAC*AGGAAA*CAGCT ATGACC

**-35** **-10** **RBS** **start**

**GI 1.2** (SEQ ID NO. 17)

GCCC **TTGACG** ATGCCACATCCTGAGCA **AATAAT** TCAACCACTAATTGTGAGCGGATAACA

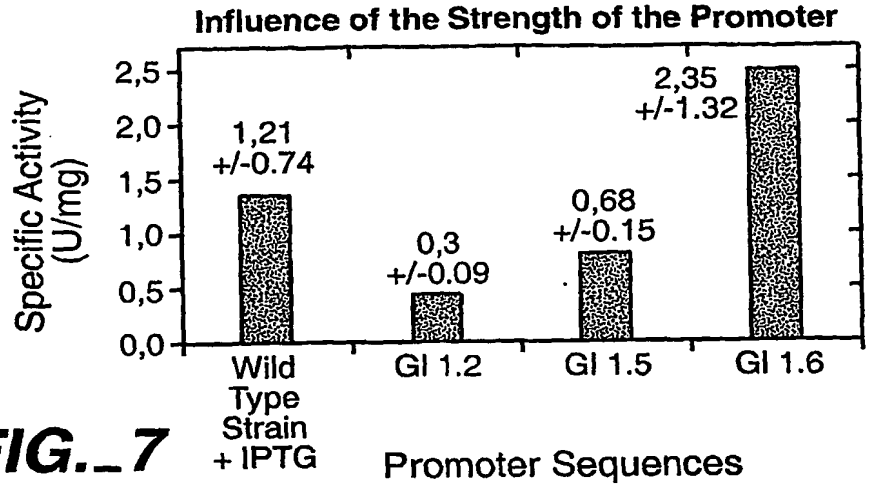


FIG._7

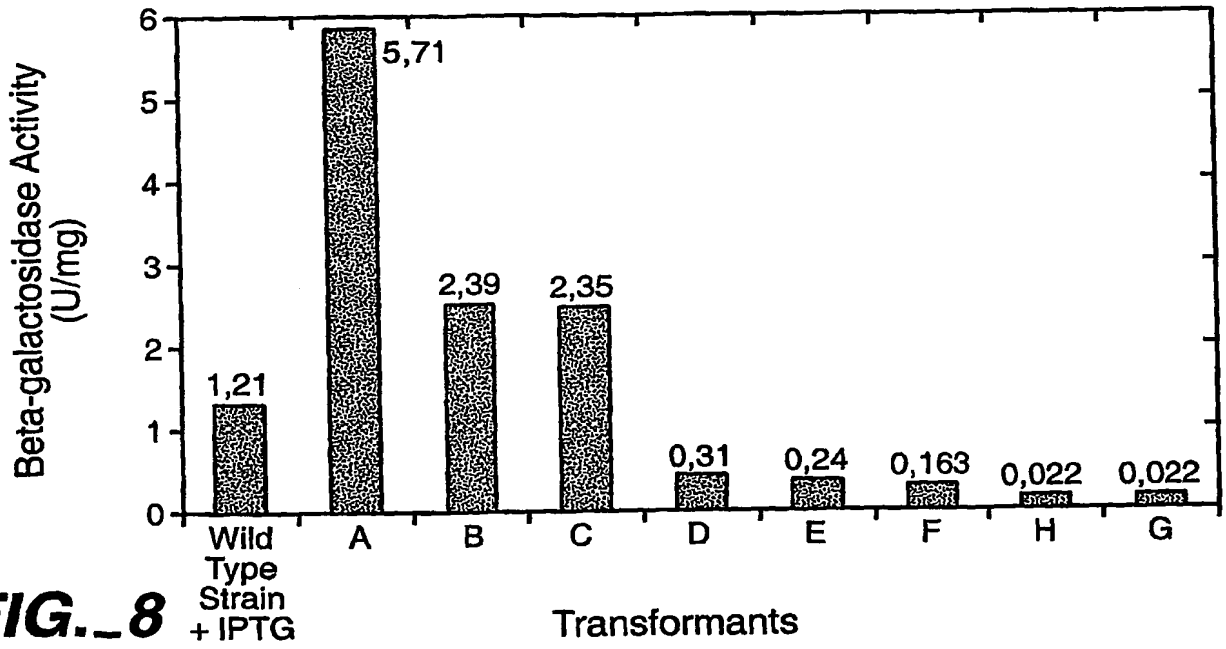


FIG._8

| Transformants | Stabilizing Sequence | RBS Sequence | Specific Activity (U/mg) |
|---|---|---|---|
| mLac RNA 1 | GGTCGAG | AAGGAGGAAA | 5.71 |
| mLac RNA 2 | GGTGGAG | AAGGAGGAAA | 11.04 |
| mLac RNA 3 | CCTCGAG | AAGGAGGAAA | 18.44 |
| mLac RNA 4 | GGTGGAC | AAGGAGGAAA | 7.3 |
| mLac RNA 5 | GCTGGAC | AAGGAGGAAA | 4.11 |
| Wild-type Strain +IPTG | NO | AGGAAA | 1.21 |

FIG._9